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SYNTHESIS OF RIBONUCLEOSIDES AND DIRIBONUCLEOSIDE PHOSPHATES CONTAINING 2-CHLOROETHYLAMINE AND NITROGEN MUSTARD RESIDUES.

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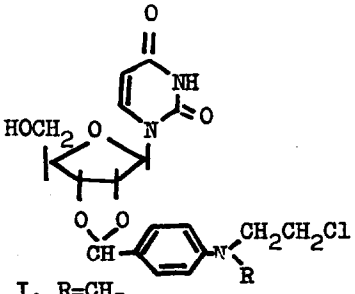
One of the main problems of biopolymers biochemistry is to find the sites responsible for their biological activity. Modification of a monomer unit of the polymer chain with some reagent and subsequent study of the activity of the modified biopolymer is one of the possible approaches to elucidate the role of this unit in the activity under investigation.

To realize this approach it is necessary to modify selectively determined monomer unit of the biopolymer molecule without touching other similar residues. Such modification sometimes appears possible due to different position of the residue in the secondary or tertiary structure of the biopolymer.

A more general approach would be provided by reagents, specially devised to discriminate between similar monomer units having different intramolecular environment due to

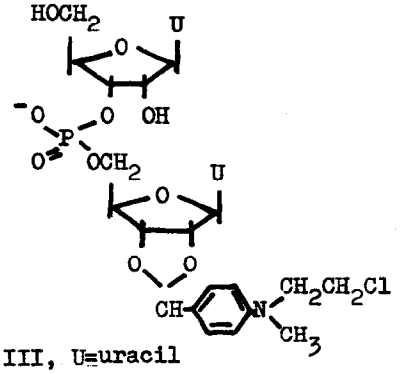
interaction with neigbour lying residues of the polymer chain. In the case of nucleic acids it seems promising for the purpose to obtain compounds, containing a reactive group bound to oligonucleotide residue capable of specific base pairing with the complementary nucleotide sequence.

The present paper is concerned with the synthesis of nucleoside and dinucleoside phosphate derivatives as the first reagents of this type. The compounds obtained are 2',3'-O-[4-(N-2chloroethyl-N-methylamino)-benzylidene]-uridine (I), 2',3'-O-[4-bis-(N-2-chloroethyl)-amino-benzylidene]-uridine (II) and uridylyl-(3'→5')-2',3'-O-[4-N-2-chloroethyl-N-methylamino)-benzylidene]-uridine (III).



I, R=CH_3

II, R=CH_2CH_2Cl



III, U=uracil

Treatment of uridine with 2-3 moles of 4-(N-2-chloroethyl-N-methylamino)-benzaldehyde (IV) or 4[-N-bis-(2--chloroethyl)-amino]-benzaldehyde (V) in the presence of 2,2-dimethoxypropane and less than one equivalent hydrogen chloride in dimethylformamide according to /1,2/ or of 2-3 equivalents of trichloroacetic acid according to

/3/ resulted in the compounds I and II respectively. Yield of I was 70%, m.p. 164–166°; after recrystallisation from mixture benzene–ethanol 4:1 m.p. 197–198° perhaps because of transformation into another diastereoisomere (4). ε_{max} at 260 mμ $36{,}1.10^3$ (dioxane). R_f 0,82 (system A*). Yield of II was 36%; m.p. 150–152°; ε_{max} at 262 mμ $23{,}1.10^3$ (dioxane). The UV-absorption spectra of I and II are the sums of those of uridine and diethylacetales of IV and V, respectively.

Compounds I and II are rather stable in organic solvents and in aqueous media in the pH interval from 5 to 9. In diluted acids, compounds I and II are readily hydrolysed to afford equimolar amounts of uridine and IV or V, respectively. At pH 2 and at 20° compound I is decomposed within 40 min, compound II- within 8min. In alkaline medium the compounds afford ionic chlorine. Besides Cl, I affords a compound with R_f 0,72 in system A.

Esterification of pyridinium 2',5'-di-O-acetyluridine-3'-phosphate with the 2–4 moles of I in pyridine in the presence of dicyclohexylcarbodiimide according to /5,6,7/ resulted in 2',5'-di-Oacetyluridylyl-(3'→5')-2',3'-O-[4-N-2--chloroethyl-N-methylamino)-benzylidene]-uridine (VI), R_f 0,72 in system B**).

Removal of acetyl protecting groups to obtain III

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*) System A: isopropanol - 25% aqueous ammonia - water 7:1:2.

**) System B: 1 M CH_3COONH_4 - ethanol 7:3, pH 7,5.

by treatment of VI with concd. aqueous ammonia resulted also in quantitative fission of the ionic chlorine. Treatment with 0,18 N hydroxylamine solution in 0,7 N NaOH for 1,5 minutes leads to quantitative deacetylation of VI, of 2',5'-di-O-acetyluridylic acid and of tetra-O-acetyluridylyl-(3'→5')-uridine (VII), as it was reported earlier for acetyl-tRNA /8/. A minor amount of ionic chlorine is splitted off during this procedure.

Compound VII has been prepared by treatment of uridylyl-(3'→5')-uridine aqueous solution with acetic anhydride in dimethylformamide at constant pH 7,0 according to /9/. R_f of VII 0,67 in system B.

It is possible also to deacetylate the compounds simultaneously with chromathography of the reaction mixture on DEAE-cellulose (bicarbonate form) using linear gradient of triethylammonium bicarbonate (from 0 to 0,2 N). The eluate was stored at +5°for about 24 hours till negative hydroxamate reaction /8/.

Compound III was obtained from VI by the above methods. The yield of III was 28%, R_f 0,46 in system A, 0,70 in B; III was purified by paper chromathography on Whatman 3 MM in system A. (Found %: Cl 4,8 , $C_{28}H_{35}ClN_6O_{14}P$. Calculated %: Cl 4,9); UV-absorption spectrum of the compound corresponded to sum of those of uridylic acid and III, ε_{max} at 263 mμ $34{,}5 \cdot 10^3$ (water).

Compound III was obtained also by treatment of uridylyl-(3'→5')-uridine with 4 moles of IV in dimethylformamide in the presence of 2,2-dimethoxypropane and two equ-

ivalents of trifluoroacetic acid for 60 hours at room temperature. Yield of triethylammonium salt III was 66%. The compound was chromathographycally homogeneous. Ratio found: uridylyl-uridine:IV- 1:1,3; uridylic acid:I- 1:1,1

Degradation of III in 0,3N aqueous alkali afforded uridylic acid and compound with R_f 0,72 in system A. The latter compound was obtained also from I under the same conditions. Cleavage of III with pancreatic RNAase resulted in uridine-3'-phosphate and I. The degree of isomerisation as revealed by hydrolysis with RNAase was 1% for the product obtained by the first of the above two methods and 7% for the product obtained by the second method. Treatment of III with 0,01 N hydrochloric acid or 5% acetic acid afforded equimolar amounts of uridylyl--uridine and IV. At the same time, the UV-absorption spectrum of III changed from the sum of those of uridylyl--uridine and acetal of IV with λ_{max} 263 mμ to the sum of UV-spectra of uridylyl- uridine and aldehyde IV with λ_{max} 260 mμ and 350 mμ.

The compound I reacts with guanosine and tRNA at pH 7,8. These results and synthesis of other 2-chloroamine derivatives of this type involving oligonucleotides, will be reported latter.

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